

Characteristics of Graft-Versus-Host Disease after Lewis-to-Brown-Norway Rat Small Bowel Transplantation

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WE REPORTED previously that Brown-Norway (BN) rats (but no other strain) developed fatal graft versus host disease (GVHD) after fully allogeneic small bowel transplantation (SBTx) under a short course of FK506 treatment.^{1,2} This study was carried out to compare the acute GVHD observed after SBTx to that of a classical model of acute GVHD induced by bone marrow transplantation (BMTx).

MATERIALS AND METHODS

Animals

Male Lewis (LEW, RT1A^b) and Brown-Norway (BN, RT1Aⁿ) rats weighing 200 to 300 g, purchased from Harlan Sprague-Dawley (Indianapolis, IN), were used for donors and recipients, respectively.

SBTx

Orthotopic SBTx with portocaval drainage was performed as described previously.³ The recipients were treated with intramuscular FK506 at a dose of 0.64 mg/kg/d on days 0 to 13.

BMTx

A total of 50×10^6 bone marrow cells and 40×10^6 splenocytes obtained from LEW were infused to total body irradiated (1000 rads) BN recipients via the penile vein. No other immunosuppression was used for BMTx recipients.

Tests for the Recipients with GVHD

Flow Cytometric Analysis. The spleens were taken from the recipients of SBTx or BMTx 2 to 6 days after the onset of GVHD for two-color flow cytometric analysis. Splenocytes were stained with McAb 163, which is specific for the RT1A^b antigens on LEW, or with McAb 163, which is specific for the RT1Aⁿ antigen on BN (gift from H.W. Kunz, Department of Pathology, University of Pittsburgh, Pittsburgh, Pa) in conjunction with McAbs to lymphocyte lineage markers (Serotec, Oxford, England), including W3/25 (rat CD4 and macrophages), OX8 (rat CD8 and some NK cells), R7.3 ($\alpha\beta$ TCR, rat T cells), OX33 (rat B cells) and 3.2.3. (rat NK cells, gift of W. Chambers, Pittsburgh Cancer Institute, Pittsburgh, Pa).

Direct Immunofluorescence. Recipient kidneys were removed 2 to 6 days after the onset of GVHD. Cryostat sections were stained with FITC-conjugated goat antirat IgG (Sera Laboratory, Westbury, NY) or IgM (Jackson, West Grove, Pa) to detect rat immunoglobulin binding to the renal glomerulus.

RESULTS

Clinical Course after BMTx and SBTx

As reported previously,¹ all six SBTx recipients developed clinical signs of acute GVHD (such as skin rash, hair loss,

and body weight loss) 22 to 27 days after grafting (9 to 14 days after discontinuation of FK506) and died with a median survival of 32 days. All four BMTx recipients showed similar signs of acute GVHD after 6 to 7 days and died between 14 and 16 days after grafting.

Determination of the Phenotype

Flow cytometric analysis of the splenocytes from the small bowel recipients with acute GVHD revealed that only $3.3 \pm 1.6\%$ lymphocytes were of donor (LEW) phenotype, in which CD4⁺ cells were dominant ($2.7 \pm 1.7\%$). Conspicuous changes in the remaining $95 \pm 2.2\%$ recipient cell populations were an increase in OX33⁺ B cells ($62.9 \pm 7.0\%$ vs $53.4 \pm 2.6\%$ in normal BN) and a decrease of $\alpha\beta$ TCR⁺ cells ($21.1 \pm 3.3\%$ vs $39.8 \pm 1.1\%$ in normal BN). NK cell population (3.2.3⁺) remained at low levels ($1.8 \pm 0.7\%$), as in normal BN ($1.8 \pm 0.4\%$). In contrast, splenocytes of BMTx GVHD animals were totally of donor phenotype, in which an increase of NK 3.2.3⁺ cells ($13.6 \pm 3.2\%$) and a decrease of OX33⁺ B cells ($4.8 \pm 0.6\%$) was significant when compared to those of a normal LEW rat.

Immunofluorescence

Linear to granular IgG deposits in the glomerular capillary loops were evident in all SBTx GVHD animals ($n = 4$), but only faint glomerular capillary loop deposition was seen in the BMTx recipients with acute GVHD ($n = 4$). Faint granular IgM deposition was present in all four SBTx recipients but negative in the BMTx animals.

DISCUSSION

A small number of donor lymphocytes had been found in animals showing clinical signs of GVHD after LEW to BN SBTx under FK506.¹ This study provides a detailed flow cytometric analysis of the splenocyte populations in these

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animals. The majority of donor cells, which consisted of 3% to 4% of total splenocytes, were mostly CD4⁺ T cells. An associated change in the components of recipient phenotype cells was a significant increase of B cells accompanied by a reduction of the T-cell population. This profile was different from that seen after BMTx GVHD, which is induced by an unbalancing of the donor-recipient immunologic interface; splenocytes of this group were all of donor origin and NK cells represented a greater percentage of the total when compared to normal controls.³

BN rats are highly susceptible to drug-induced autoimmunity, characterized by proliferation of B and CD4⁺ T cells, hyperimmunoglobulinemia, and the production of autoantibodies.⁴ This study suggests that acute GVHD after SBTx, which is also BN specific,² elicits a similar autoimmune response. More precise characterization of the renal immunoglobulin deposits is under way. Similarities between a chronic or lupuslike GVHD induced in nonirradiated F1 hybrid by an injection of parental lymphocytes can also be seen and results from recipient B-cell hyperplasia and production of lupuslike autoantibodies. In

this latter model, persistence of donor alloreactive CD4⁺ T cells (3% to 4%) is required for disease induction.⁵

The phenotypic changes seen in SBTx recipients with acute GVHD suggest that the disease may be the result of cooperation between donor T-helper cells and recipient B cells, similar to lupuslike GVHD. The glomerular deposition of linear and granular rat IgG by immunofluorescence supports the hypothesis that the formation of autoantibodies plays a role in the development of GVHD after SBTx in the BN rat.

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